PB82-207838

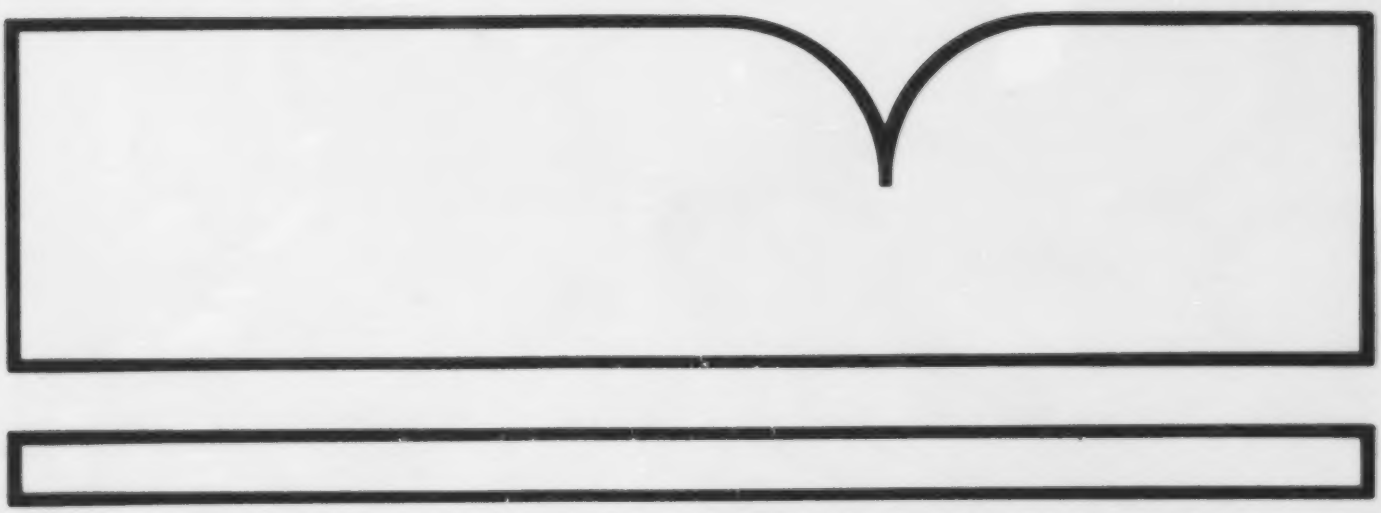
Rosette Glands in the Gills of the Grass Shrimp 'Palaemonetes Pugio'. II. Premolt Ductule Reformation: Replacement of Ciliary Processes by Cytoplasmic Processes in Relation to Gland Maturation

University of West Florida
Pensacola

Prepared for

Environmental Research Lab.
Gulf Breeze FL

1982



U.S. Department of Commerce
National Technical Information Service
NTIS

ERL,GB 0224

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO.	2.	3.
EPA-600/J-82-149	Journal Article	PB82-207838

4. TITLE AND SUBTITLE
ROSETTE GLANDS IN THE GILLS OF THE GRASS SHRIMP PALAEMONETES PUGIO. II. PREMOLT DUCTULE REFORMATION: REPLACEMENT OF CILIARY PROCESSES BY CYTOPLASMIC PROCESSES IN RELATION TO GLAND MATURATION

5. REPORT DATE
April 1982

6. PERFORMING ORGANIZATION CODE

7. AUTHOR(S)
D.G. Doughtie and K. R. Rao

8. PERFORMING ORGANIZATION REPORT NO.

9. PERFORMING ORGANIZATION NAME AND ADDRESS
Faculty of Biology
University of West Florida
Pensacola, FL 32504

10. PROGRAM ELEMENT NO.

11. CONTRACT/GRANT NO.
R807417

12. SPONSORING AGENCY NAME AND ADDRESS
U.S. Environmental Protection Agency
Environmental Research Laboratory
Office of Research and Development
Gulf Breeze, FL 32561

13. TYPE OF REPORT AND PERIOD COVERED

14. SPONSORING AGENCY CODE
EPA/600

15. SUPPLEMENTARY NOTES
Journal of Morphology 171:69-77

16. ABSTRACT

Events associated with premolt reformation of the cuticularized ductule in the underdeveloped (immature) branchial rosette glands, which are common in the gills of small (14-18 mmn, total length) grass shrimp, are described and contrasted with the events of ductule reformation in the fully developed (mature) rosette glands most common in larger shrimp. Cytoplasmic processes comparable to those in mature glands, but relatively few in number and originating only from the secretory cells, are seen together with ciliary processes in some immature glands. The relative abundance of cytoplasmic processes in the mature glands, coupled with the observation that transitory ciliary processes occur in immature glands but not in mature glands, suggests that during glandular maturation transitory ciliary processes are replaced by transitory cytoplasmic processes.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

REPRODUCED BY
NATIONAL TECHNICAL
INFORMATION SERVICE
U.S. DEPARTMENT OF COMMERCE
SPRINGFIELD, VA 22161

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
Release to public	unclassified	11
	20. SECURITY CLASS *(This page)* unclassified	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

EPA-600/J-82-149
Journal Article

JOURNAL OF MORPHOLOGY 171:69-77 (1982)

Rosette Glands in the Gills of the Grass Shrimp *Palaemonetes pugio*. II. Premolt Ductule Reformation: Replacement of Ciliary Processes by Cytoplasmic Processes in Relation to Gland Maturation

DANIEL G. DOUGHTIE AND K. RANGA RAO
Faculty of Biology, University of West Florida, Pensacola, Florida 32504

ABSTRACT The events associated with premolt reformation of the cuticularized ductule in the underdeveloped (immature) branchial rosette glands, which are common in the gills of small (14–18 mm, total length) grass shrimp, are described and contrasted with the events of ductule reformation in the fully developed (mature) rosette glands most common in larger shrimp. In immature rosette glands, two ciliary processes emerge from each of the component secretory cells and ascend into the basal luminal region of the old ductule. Subsequently a new ductule is formed around the old ductule, and the ciliary processes disappear, either because of degeneration or retraction. The transitory ciliary processes appear to prevent the old ductule from collapsing during the formation of a new ductule. Such transitory ciliary processes, however, are not found in association with premolt ductule reformation in the mature rosette glands; in their place are seen a number of microvilli-like cytoplasmic processes, which emanate from the apices of the secretory cells and from the channels of the central cell. These cytoplasmic processes in mature glands, like the ciliary processes in immature glands, are transitory and appear to prevent the collapse of the old ductule.

Cytoplasmic processes comparable to those in mature glands, but relatively few in number and originating only from the secretory cells, are seen together with ciliary processes in some immature glands. The relative abundance of cytoplasmic processes in the mature glands, coupled with the observation that transitory ciliary processes occur in immature glands but not in mature glands, suggests that, during glandular maturation, transitory ciliary processes are replaced by transitory cytoplasmic processes.

Previous studies on premolt ductule reformation in the exocrine glands of Crustacea have been restricted to an ultrastructural study of the events in the tricellular dermal gland in *Palaemonetes* (Doughtie and Rao, '79). In this gland, as in the developing exocrine glands of insects (Barbier, '74, '75; Berry and Johnson, '75; Selman and Kafatos, '75; Bitsch and Palevody, '76; Sreng and Quennedy, '76), temporary ciliary structures were shown to be associated with ductule formation. Furthermore, these studies have indicated that the temporary ciliary processes probably serve as a template around which a new cuticular ductule is formed.

This report describes the events associated with premolt ductule reformation in the branchial rosette glands of the grass shrimp, *Palaemonetes pugio*. The general morphology, ultrastructural features, and molt-related secretory activities of these rosette glands have been described in the first paper of this series (Doughtie and Rao, '82).

MATERIALS AND METHODS

The procedures employed in this study were identical to those described in the first paper of this series (Doughtie and Rao, '82).

RESULTS

The following generalizations, abstracted from the results reported in the first paper of this series (Doughtie and Rao, '82), provide the background information on the rosette glands

whose premolt ductule reformation is described in this report. The pleurobranchiate gills of *Palaemonetes* contain two types of rosette glands (type A and type B), each of which is composed of a variable number of secretory cells, a central cell, and a canal cell, as well as a cuticular ductule, which carries secretions to the branchial surface.

According to the number of secretory cells, the degree of cytoplasmic specialization in the secretory cells, and the diameter of the ductule, the two gland types can be distinguished as being either underdeveloped (immature) or well developed (mature). For example, the immature type A glands possess fewer secretory cells (< 11) than do the mature type A glands (14–17 cells); the immature type A rosette gland secretory cells possess fewer secretory granules and a less well-developed rough endoplasmic reticulum than do the mature type A secretory cells; and the diameter of the ductule in immature glands (0.3–0.6 μm) is less than in mature glands (0.8–1.8 μm). Finally, the immature rosette glands (types A and B) are most common in the gills of smaller shrimp (14–18 mm, total length), whereas the mature rosette glands (types A and B) are predominant in the gills of larger (24–28 mm) shrimp.

Premolt ductule reformation

Insofar as the events associated with ductule reformation are concerned, no differences were seen between type A and type B glands, although, within both gland types, differences were noted between immature and mature glands. These differences are described below. To minimize redundancy, the majority of the accompanying micrographs pertain to the events of ductule reformation only in the immature and mature type A rosette glands.

Occurrence of ciliary processes during premolt ductule reformation in the immature rosette glands. At apolysis, the apex of each of the rosette gland secretory cells is characterized by the presence of two narrow cylindrical invaginations (≅ 1.5 μm long; 0.3 μm diameter) or sheaths, which communicate with the anastomosing extracellular channels of the central cell. A ciliary basal body (Figs. 1, 4) is situated at the base of each sheath, where it is anchored to the subjacent cytoplasm by short (0.3 μm) rootlets with a periodicity of 600 Å. A ciliary process develops from each basal body and ascends through the sheath (Figs. 1, 2) into the extracellular channels (Fig. 3) and intima-lined vent (Fig. 4) of the central cell, and finally into the preexisting cuticular ductule of the canal cell (Fig. 5). Near the basal body, the ciliary process is characterized by a 9 + 0 arrangement of microtubules (Fig. 4) and a diameter of between 0.19 and 0.26 μm. However, before emerging from the secretory cell sheath the microtubule doublets become disorganized and numerically reduced (Fig. 2), and at more distal levels, as within the ductule, only a small percentage of the original microtubules persist (Fig. 6). Perhaps as a result of this reduction in the number of microtubules, the diameter of the apical regions of the ciliary processes is less (≅ 0.13–0.16 μm) than that of the basal region. The tips of the ciliary processes appear electron-dense (Fig. 7).

Following the growth of the ciliary processes into the preexisting ductule, epicuticular plaques are formed along the canal, which is that surface of the canal cell plasmalemma which envelops the ductule (Fig. 6). As the cuticular plaques become more numerous, they coalesce to form a continuous sheet, which constitutes the wall of the new ductule. Subsequent to the reformation of the new ductule the ciliary processes degenerate or retract, but the old ductule into which they ascended is not sloughed until ecdysis.

In some of the immature rosette glands, the growth of ciliary processes is accompanied by a commensurate growth of occasional microvilli-like, cytoplasmic processes originating from the invaginated apical plasmalemma of the secretory cells (Fig. 8). These processes (0.07–0.13 μm diameter) contain microfilaments that are parallel to the long axis of the processes. Usually the paths taken by these growing processes are restricted to the extracellular channels of the central cell, and in some instances they appear to cross over and into the apical invagination of an adjacent secretory cell. Figure 9 appears to demonstrate that at least some cytoplasmic processes ascend into the vent of the central cell.

Occurrence of cytoplasmic processes during premolt ductule reformation in the mature rosette glands. At apolysis, the secretory apparatuses of mature rosette gland secretory cells become occluded by microvilli-like cytoplasmic processes (0.10–0.12 μm diameter). These processes contain microfilaments and emerge initially only from the plasmalemmal surfaces of the secretory apparatuses (Figs. 10, 11). The processes begin to ascend into the extracellular channels and vent of the central cell at the same time when epicuticular plaques

Abbreviations

bb, basal body
C, central cell
cc, canal cell
ch, extracellular channels of central cell
cp, ciliary process
d, ductule
i, intima of central cell vent
nd, new ductule
ni, new intima
od, old ductule
oi, old intima
p, cytoplasmic process
r, ciliary rootlets
sa, secretory apparatus
SC, secretory cell
v, central cell vent

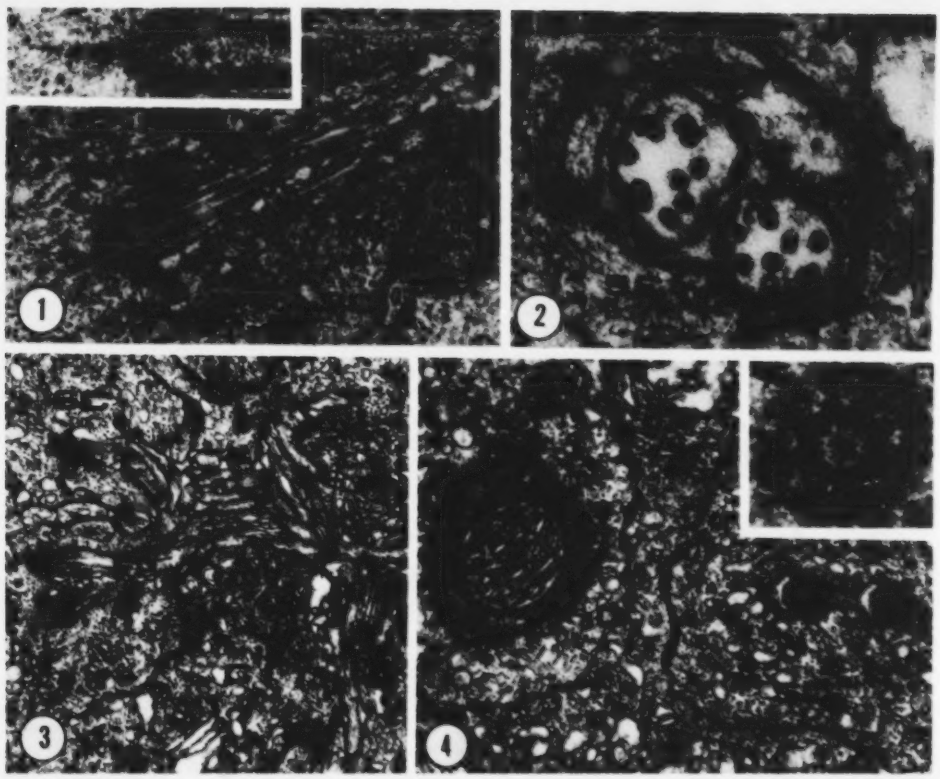
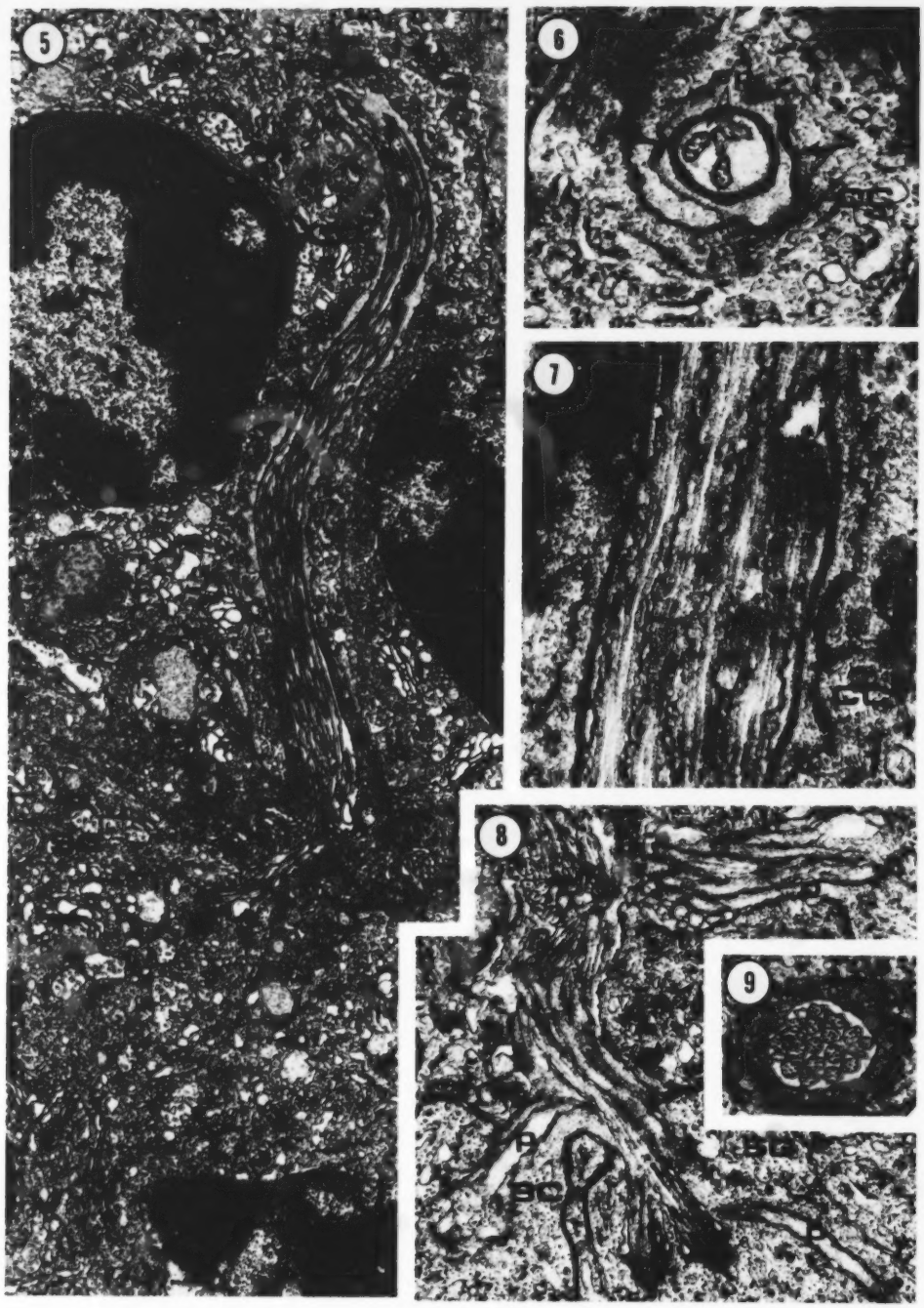


Fig. 1. A ciliary structure possessing rootlets (r), a basal body (bb), and a ciliary process (cp) is situated within a cylindrical invagination of a type A gland secretory cell (SC) apex during apolysis. × 30,000. Inset. Higher magnification of the basal body and rootlets. × 60,000.

Fig. 2. Cross section of two ciliary processes at a distance of approximately 1 μm from the type A gland secretory cell (SC) apex. Notice that the microtubule doublets, which characterize the basal region of the ciliary process, become disorganized (arrow) and reduced in number, farther away from the ciliary basal body. × 100,000.

Fig. 3. The ciliary processes (cp) from each pair of ciliary structures within the apical cytoplasm of immature type A rosette gland secretory cells (SC) pass into the central cell (C) extracellular channels (ch) at apolysis. × 15,000.

Fig. 4. Section through the core of an immature type A rosette gland showing the bases of two ciliary structures in a secretory cell (SC) and the closely packed ciliary processes, from other secretory cells, passing through the vent (v) of the central cell (C). One of the ciliary structures has been cross-sectioned at the junction between the basal body and the ciliary process showing a 9 + 0 arrangement of microtubule doublets (arrow). × 20,000. Inset: High-magnification cross section through the basal body, in which microtubule triplets are visible (arrow). × 70,000.


5
6
7
8
9
P
BC
BC
P

appear at the plasmalemmal surface of the canal that envelops the preexisting ductule (Fig. 10, inset). During the later stages of epicuticle formation, when adjacent epicuticular plaques coalesce to form a continuous cuticular sheet, the vent of the central cell is filled with cytoplasmic processes (Fig. 12); by this time, the processes are seen to originate not only from the secretory cell apices, but also from the plasmalemmal surfaces of the central cell extracellular channels (Figs. 12, 13). The processes continue to lengthen and ascend through the vent into the old cuticularized ductule of the canal cell (Figs. 14, 15). The presence of many ascending processes effectively maintains the luminal dimensions of the old ductule as the new ductule is formed around the latter (Fig. 15). The ultimate distance covered by the elongating cytoplasmic processes is not known. Although the processes have been observed within the old ductule at sites near the gland periphery, their presence in the more apical regions of the ductule between the main body of the rosette gland and the branchial surface could not be demonstrated.

Subsequent to the formation of the branchial inner epicuticle, the cytoplasmic processes begin to disappear, either as a result of retraction or degeneration (Fig. 16), thereby leaving the extracellular channels and newly formed cuticular ductule open for the passage of glandular secretions. Following the complete disappearance of the cytoplasmic processes from the vent of the central cell, the fibrous intima that lines the vent frequently appears bilaminar (Fig. 17), the outer layer being the basal region of the new ductule, and the inner layer being the basal region of the old ductule. The latter inner layer remains attached to the old ductule within the canal cell (Fig. 18), and is sloughed at ecdysis.

Finally, it should be noted that ciliary processes are virtually absent in the mature type A and type B rosette glands during premolt ductule reformation. However, as demonstrated in Figure 19, basal bodies are occasionally associated with the secretory apparatuses of mature glands although ciliary rootlets are not apparent. It is likely that these basal bodies are vestiges of the ciliary elements that are predominant in the immature glands.

DISCUSSION

Occurrence of temporary ciliary processes in immature rosette glands

In the previous studies on ductule reformation in arthropod exocrine glands, it has been shown that the preexisting ductule is sloughed into the exuvial space prior to the formation of a new ductule (Lai-Fook, '73; Doughtie and Rao, '79). These studies have also shown that the re-forming cuticular ductules, like the original ductules in insect glands undergoing morphogenesis (Barbier, '74, '75; Berry and Johnson, '75; Selman and Kafatos, '75; Bitsch and Palevody, '76; Sreng and Quenedy, '76), are formed directly around temporary ciliary or ciliary-like processes; thus, it is generally believed that temporary ciliary processes in arthropod exocrine glands function as a true template. However, the ciliary processes that develop in the immature rosette glands of *Palaemonetes* do not function as a true template. Specifically, the old preexisting ductule, into which the ciliary processes ascend, is not sloughed until ecdysis. As a result, it is the old ductule that appears to function as a template for the re-forming ductule. This being the case, it is important that the full diameter of the old ductule be maintained so that the diameter of the new ductule can be increased. Such an in-

Fig. 5. Rare section through immature type A rosette gland showing the ascent of numerous ciliary processes, originating from the secretory cell (SC) apices, through the central cell (C) extracellular channels (ch) and into the old ductule of the canal cell (CC). Extremely close examination of this micrograph shows small cuticular plaques over the canal cell plasmalemma (arrows). These plaques later coalesce to form the new ductule. × 9,500.

Fig. 6. Cross section of the old ductule in the canal cell (CC) near the periphery of an immature type A rosette gland. Notice the presence of cuticular plaques (arrow) adjacent to the canal cell plasmalemma which will form the new ductule. Only a few ciliary processes (cp), with a reduced number of microtubules, ascend to or beyond the gland periphery. × 30,000.

Fig. 7. Electron-dense tip (arrow) of a ciliary process as it ascends through the old cuticular ductule (d) of the canal cell (CC). Type A rosette gland. × 75,000.

Fig. 8. Section through the core of an immature type A rosette gland demonstrating the presence of microvilli-like cytoplasmic processes (p) emerging from the secretory cell apices. × 20,000.

Fig. 9. Cross section of central cell vent region of an immature type A rosette gland during early premolt. In addition to the ciliary processes, several microvilli-like processes (arrows) ascend toward the ductule. × 20,000.

i
10
11
13
SC
12
14
15

crease in ductule diameter is desirable, for gland maturation involves an increase not only in the number of secretory cells but also in the amount of secretion that must pass to the branchial surface (Doughtie and Rao, '82). It is therefore believed that the function of the ascending ciliary processes is to prevent the old ductule from collapsing during the re-formation of a new ductule. This potential for ductule collapse presumably during periods of negligible secretory activity has already been demonstrated (Doughtie and Rao, '82).

The phenomenon of ductule reformation in the immature rosette glands was usually associated only with temporary ciliary processes. In some glands, however, the growth into the old ductule of the temporary ciliary processes was accompanied by the growth of a relatively small number of cytoplasmic processes. Even more interesting was the fact that in the more fully developed mature rosette glands, ductule re-formation occurred in the complete absence of ciliary processes.

Occurrence of temporary cytoplasmic processes in mature rosette glands

Temporary cytoplasmic processes, which lack both microtubules and a basal body (or "centriole"), have been described in only a few of the arthropod exocrine glands in which ductule formation and re-formation were observed (Lai-Fook, '73; Doughtie and Rao, '79). In the tricellular dermal gland of *Palaemonetes* (Doughtie and Rao, '79) the temporary cytoplasmic processes evolved during premolt, soon after the development of temporary (presumably template-forming) ciliary processes. As in the rosette glands described here, the cytoplasmic processes in the tricellular dermal gland developed not only from the secretory cell but also from the plasmalemma of the intercalary "hillock" cell (comparable to the central cell in the rosette gland). It was suggested that these processes contributed to the formation of certain extracellular channels, including the secretory apparatus and the "locular complex" (Doughtie and Rao, '79). In contrast to their independent development in the rosette and tricellular glands of *Palaemonetes*, the cytoplasmic processes in *Calpodes* Verson's glands were presumed to evolve directly from previously formed, ciliary-like processes (Lai-Fook, '73). In the Verson's gland, it was suggested that the cytoplasmic processes maintained the shape of certain extracellular invaginations during the deposition of the cuticular ductule and saccule.

The function of the temporary cytoplasmic processes in *Palaemonetes* rosette glands appears to be different from the roles ascribed to similar processes in either the tricellular gland or the Verson's gland. Specifically, there is a striking similarity between both the temporary ciliary processes found in immature rosette glands and the independently evolved cytoplasmic processes that occur in mature rosette glands. For example, both types 1) develop during the early premolt stages of ductule reformation, 2) are similar in terms of their dimensions and degree of ascent into the preexisting ductule, and 3) disappear subsequent to the re-formation of the new ductule. Because of these similarities, it is tempting to suggest that the cytoplasmic processes function in a

Fig. 10. Section through the core of a mature type A rosette gland in which microvilli-like cytoplasmic processes (p) are beginning to emerge from the secretory cell (SC) apices and ascend into the vent (v) of the central cell (C) toward the canal cell ductule (d). × 8,000. Inset: High magnification of point where the intima (i) of the central cell vent merges with canal cell ductule (d). Notice that concurrent with the growth of cytoplasmic processes, cuticular plaques are already operating above the canal cell plasmalemma (arrows). These will later coalesce to form the new ductule. × 22,000.

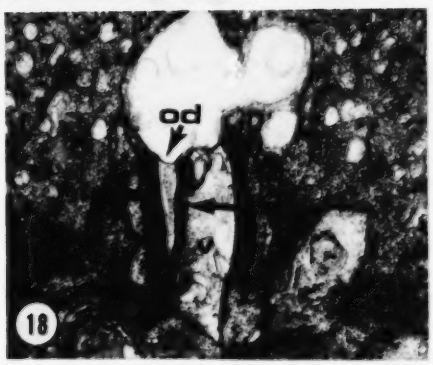
Fig. 11. The apices of two adjacent secretory cells (SC) in a mature type A rosette gland, protruding into the fibrillar cytoplasm of the central cell (C) during early premolt. Notice the emergence of numerous cytoplasmic processes (arrows) from the plasmalemma of the secretory cells. × 20,000.

Fig. 12. Rare section through the core of a mature type A rosette gland during early premolt, showing the virtual occlusion of the central cell (C) extracellular channels (ch) and vent (v) by growing, microvilli-like cytoplasmic processes. Notice that whereas most of the processes emerge from the secretory cell apices (SC), there are some that seem to originate from the central cell (arrows). × 16,000.

Fig. 13. Section through an extracellular channel (ch) of the central cell (c) showing cytoplasmic processes originating from the secretory cell apex as well as other more electron-dense processes (arrow) that appear to emanate from the central cell plasmalemma. × 20,000.

Fig. 14. Sagittal section of ductule passing through the canal cell (CC) near the periphery of a type A rosette gland during early premolt. Notice that cytoplasmic processes nearly fill the old ductule and that the new ductule is nearly formed (arrow) around the latter. × 10,000.

Fig. 15. Cross section of the ductule in a mature type A rosette gland during early premolt. Notice that the cytoplasmic processes (p) appear to maintain the lumen of the old ductule (od) in an expanded state, and also note that the new ductule (nd) closely conforms to the dimensions of the old ductule. × 20,000.



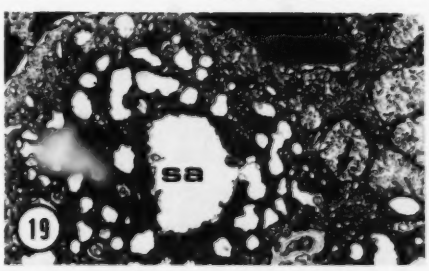


Fig. 16. Subsequent to the formation of branchial epicuticle and the new ductule (d), the cytoplasmic processes (p) within the old ductule (od) become sparse either as a result of retraction or degeneration. Central cell, C. × 13,500.

Fig. 17. Cross section through the vent region (v) of a type A rosette gland central cell after degeneration of the cytoplasmic processes has occurred. Notice that the fibrous intima of the vent is bilaminar (arrows). The new, outermost layer of the intima has been reformed directly around the older intima. Canal cell, CC. × 20,000.

Fig. 18. Sagittal section of vent region (v) in mature type B rosette gland during late premolt. Notice the partial separation of the old intima (oi) from the newly formed intima (ni) of the central cell (C) and the continuity of the former with the old ductule (od). × 10,000.

Fig. 19. Section showing the secretory apparatus (sa) of a mature type B rosette gland just prior to ecdysis. Notice the vestigial basal body (arrow) in close apposition to the secretory apparatus. In mature glands the basal bodies do not form ciliary processes during ductule reformation. × 7,000.

similar capacity as the ciliary processes in underdeveloped glands; that is, to prevent the walls of the old ductule from collapsing during the formation of new ductule. In this respect it is also interesting to note that the cytoplasmic processes, as well as the ciliary processes, were never observed in that region of the ductule that resides between the main body of the rosette gland and the branchial surface. The reason why the ascent of these processes appears to be limited only to that region of the ductule within the main body of the rosette gland is not well understood. It is possible that forces that could cause ductule collapse are greatest within the main body of the rosette glands. For example, the secretory cells of the type A rosette glands appear to be engaged in accelerated synthesis of secretory granules during early premolt (Doughtie and Rao, '82). Perhaps there is a commensurate increase in secretory cell volume that might exert sufficient pressure on the adjacent canal cell and the enclosed ductule to cause the latter to collapse. Additional studies are necessary to answer this question adequately.

Another interesting feature relating to the occurrence of temporary cytoplasmic processes during ductule re-formation in mature rosette glands is that it appears that they gradually replace the ciliary processes that are found in underdeveloped glands. The gradual nature of this transition is suggested by the occurrence in some underdeveloped glands of occasional cytoplasmic processes (see Fig. 8), and by the occurrence in some well-developed mature glands of vestigial ciliary structures (see Fig. 19).

ACKNOWLEDGMENTS

This study was part of a project supported by grant CR-807417 from the U.S. Environmental Protection Agency. We are thankful to Dr. Thomas Duke, Gulf Breeze Environmental Research Laboratory, for his support and encouragement during this investigation.

LITERATURE CITED

Barbier, R. (1974) Presences de structures ciliares au cours de l'organogénèse des glandes colleteriques de *Galleria mellonella* L. (Lepidoptere, Pyralide). J. Microsc. *20*:18–19.

Barbier, R. (1975) Differenciation de structures ciliares et mise en place des canaux au cours de l'organogénèse des glandes colleteriques de *Galleria mellonella* L. (Lepidoptere, Pyralide). J. Microsc. Biol. Cell. *24*:315–326.

Berry, S.J., and E. Johnson (1975) Formation of temporary flagellar structures during insect organogenesis. J. Cell Biol. *65*:489–492.

Bitsch, J., and C. Palevody (1976) Modifications ultrastructurales des glandes vesiculaires des Machilidae (Insecta, Thysanura) au cours des cycles de mue. Zoomorphologie *83*:89–108.

Doughtie, D.G., and K.R. Rao (1979) Ultrastructure of an exocrine dermal gland in the gills of the grass shrimp, *Palaemonetes pugio:* Occurrence of transitory ciliary axonemes associated with the sloughing and reformation of the ductule. J. Morphol. *161*:281–308.

Doughtie, D.G.,and K.R. Rao (1982) Rosette glands in the gills of the grass shrimp, *Palaemonetes pugio.* I. Comparative morphology, cyclical activity, and innervation. J. Morphol. (in press).

Lai-Fook, J. (1973) The fine structure of Verson's glands in molting larvae of *Calpodes ethlius* (Hesperiidae, Lepidoptera). Can. J. Zool. *51*:1201–1210.

Selman, K., and F.C. Kafatos (1975) Differentiation in the cocoonase producing silkmoth galea: Ultrastructural studies. Dev. Biol. *46*:132–150.

Sreng, L., and A. Quennedy (1976) Role of a temporary ciliary structure in the morphogenesis of insect glands. J. Ultrastruct. Res. *56*:78–95.